Alloreactive natural killer cell therapy for multiple myeloma

Summary

Multiple Myeloma accounts for about 13% of the hematological cancers and is responsible for 0.9- 1.2% of deaths from cancer. Most people are above the age of 65 when the diagnosis is made. After patients are diagnosed with Multiple Myeloma, they are treated with a combination of frontline chemotherapy, which includes a variety of drugs. Thus far, there is no option to cure Multiple Myeloma. Therefore, the effort is directed to improve overall survival and maintaining quality of life. However, in the last decade, several drugs, like bortezomib, thalidomide and lenalidomide, have become available that due to a better anti-myeloma effect improve survival of the patients.

The precise combination of drugs to be used is depending on age and comorbidity. Eligible patients with a healthy immune status are treated with a regimen that aims for reduction in myeloma burden and after this induction treatment high dose chemotherapy will follow. An important step prior to this chemotherapeutic procedure is to isolate the patients hematopoietic stem cells (HSCs) and freeze them, since the intensive chemotherapy treatment will not only destroy tumor cells, but also healthy bone marrow stem cells that are needed to produce all blood cells. Therefore, the high dose chemotherapy will be followed by a return of the frozen HSCs, a procedure called autologous stem cell transplantation (auto-SCT). Several clinical trials have indicated that this procedure improves overall survival. Despite this intensive treatment schedule, patients will not be cured, though some patients show a long period (years) of progression free survival with good quality of life.

Allogeneic SCT (allo-SCT) is an alternative to auto-SCT. By making use of donor stem cells, one can transplant a donor immune system to the recipient patient. This procedure, that was originally developed to compensate for radiation damage by which peoples' bone marrow stem cells were destroyed, has the potential to cure malignant diseases by the so called graft (=donor) versus disease response of the immune system. However, this procedure can also induce an unwanted graft versus host effect (GVH) with detrimental effects on healthy organs, mostly skin, gut, liver and lungs. This GVH effect is due to the fact that donors that are selected will still be mismatched for (minor) histocompatibility antigens that evoke the donor immune system to respond.

Since the limitations of the clinical outcome of allo-SCT and the scarcity of accurately matched donors to perform an allo-SCT as described above on time, investigators have looked for alternative donors. The use of haploidentical donors, sharing one of the two haplotypes with the patient can considerably overcome this problem of donor availability. Every patient will usually have a donor since parents, siblings, children and some nieces/nephews will share one haplotype (a set of chromosomes with the relevant transplantation antigens, coming from the father or mother) with the patient.

Ruggeri *et al*. also showed in a landmark paper the phenomenon of enhanced graft vs acute myeloid leukemia (AML) response during haploidentical stem cell transplantation. Surprisingly, the clinical anti-tumor effect was probably due to graft versus tumor effect of the NK cells and not due to T cells. Our group has shown that haploidentical stem cell transplantation together with nonmyeloablative conditioning regimen can cure breast cancer in a 4T1 breast cancer mice model. Subsequent experiments by our group identified donor NK cells as crucial for the graft versus tumor effect during haploidentical transplantation in this model. NK cell depletion of the haploidentical graft fully abrogated the anti-tumor effect and only NK cells in the absence of donor stem cells demonstrated similar results, confirming the data of Ruggeri *et al*. that NK cells from haploidentical donors might have the potency for strong anti-tumor effects, not only in hematological tumors, but also in solid tumors.

NK cells are large granular lymphocytes, which can selectively kill virally infected cells and tumor cells without affecting healthy normal cells. Certain NK cell subsets can mount direct cytotoxic action against virally infected or tumor cells. In Chapter 2, we show the need to perform *in vitro* investigations to study interactions between NK cells and hematological tumor cells under hypoxic conditions. Furthermore, our study provides proof of concept that activating cytokines can overcome the adverse effects of hypoxia *in vitro* and shows that hypoxia is a factor to take into account when designing allogeneic NK cell based immunotherapy for MM. Experimental set ups comparable to ours will be helpful to determine the potential of novel and existing cytokines or immunomodulatory agents, to boost NK cell responses in a way that NK cells can also exert their effector function in the presence of tumor environmental factors like hypoxia. For future clinical perspectives it might be considered to combine NK cell therapy with hypoxia-targeting and pre-activation of NK cells to eliminate tumor cells in a hypoxic environment.

Immunotherapy with allogeneic NK cells offers therapeutic perspectives for Multiple Myeloma patients. In Chapter 3, we aimed to refine NK cell therapy by evaluation of the relevance of HLA-class I and HLA-E for NK-anti-myeloma reactivity. Our results show that KIR-HLA-class I and NKG2A-HLA-E interactions are highly relevant for NK cell reactivity against myeloma. For accurate prediction of *in vitro* data to patient's reality, two relevant *in vivo* realities have to be taken into account: 1) in the bone marrow myeloma cells reside under hypoxic conditions and 2) *in vivo*, myeloma cells express both HLA-class I and HLA-E. Infusion of a high number of cytokine activated alloreactive NKG2A negative, KIR-ligand mismatched NK cells may help to improve the efficacy of alloreactive NK cell therapy. Our study demonstrates that NKG2A-negative, KIR-ligand mismatched NK cells are the most potent subset for clinical application. We envision that infusion of high numbers of this subclass will enhance clinical efficacy.

In Chapter 4, we explored the activity of alloreactive Natural Killer (NK) cells after chemo-radiotherapy conditioning as a novel immunotherapy treatment option for Multiple Myeloma (MM). Using the RAG2$^{-/-}\gamma c^{-/-}$ immunodeficient disseminated MM mouse model, we studied NK cell mediated Graft versus MM (GvM). Our study illustrates the therapeutic potential and safety of NK cell therapy in MM and is an important step towards cell-based immunotherapy for MM patients without the induction of GvHD. It also establishes the RAG2$^{-/-}\gamma c^{-/-}$ MM model as a clinically relevant platform to further develop NK cell based immunotherapy. We showed the biological anti-MM potential of alloreactive NK cells against disseminated MM. In addition, we demonstrate that IL-2 activated NK cells can home to the bone marrow of MM bearing mice and that anti-MM responses occurred in a dose dependent manner. The latter suggests that infusion of a higher number of alloreactive NK cells can help to improve clinical efficacy and that patients should, preferably, be treated in remission or at least with low residual disease. Our data also demonstrate that combination therapy of alloreactive NK cells and chemo/radiotherapy could help to improve the anti-MM effect.

Success of new generation anti-MM drugs over the past decade has raised hopes for treating Myeloma. Discovery of new generation chemotherapeutics and monoclonal antibodies (MoAb's) for MM have significantly improved overall survival in the last decade. These novel drugs have demonstrated to display immunomodulatory (IMD) effects on NK cells in vitro or trigger antibody dependent cellular cytotoxicity (ADCC). We therefore hypothesized in Chapter 5 that combining these drugs with NK cell therapy may provide a successful approach for the treatment of MM. We observed that MM cells treated with an array of different IMD anti-MM drugs or MAb's, and cultured under hypoxia (1% or 0.2% O_2) were not enhancing NK cell cytotoxicity as compared to untreated cells. This study demonstrates the necessity of in vitro anti-myeloma therapeutic research at hypoxic oxygen levels representative of bone marrow environment to be able to draw the right conclusions about new possibilities.

All together, we have demonstrated in the work described in this thesis that there might be a future for donor NK cell therapy in MM patients to potentially cure them. Since NK cell therapy is a completely different approach than chemotherapy, where there is ample evidence that cancer cells residing in the hypoxic areas of tumors are therefore less responsive to these drugs, most likely combinations of treatment procedures (NK cell therapy and drugs that are active in hypoxic areas) will have the best clinical outcome since we showed here that IL-2 activated NK cells surely can kill under hypoxic conditions.